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COLLEGE OF AGRICULTURE
AGRICULTURAL EXPERIMENT STATION
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BACTERIAL BLIGHT OF GARDEN STOCKS AND ITS CONTROL BY HOT-WATER SEED TREATMENT

JAMES B. KENDRICK and KENNETH F. BAKER

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JAMES B. KENDRICK[2] AND KENNETH F. BAKER[3, 4]

INTRODUCTION

SINCE 1933 bacterial blight of the garden stock (*Mathiola incana* R. Br.)[5] has caused severe losses on commercial flower-seed ranches of California. Many young plants are killed outright, whereas others seemingly escape the seedling stage of the disease. As the plants become larger and woody, however, action of the disease is less rapid, frequently appearing as severe stem lesions, especially at or just above the ground line. The disease progresses throughout the season, eventually killing many older plants, and so severely injuring others that little or no viable seed is produced. In 1936 and in 1939 this blight caused nearly a total crop failure on some flower-seed ranches. Cultures from all stages of infection showed a yellow bacterial organism associated with the disease.

Two bacterial diseases of stocks have been reported in the literature. In 1900 van Hall (*10*)[6] reported and briefly described a bacterial disease of summer stocks[7] in Holland. This bacteriosis was considered by von Faber (*9*) to be probably the same disease of stocks as that found in Germany in 1907 and attributed by him to *Phytomonas campestris* (Pammel) Bergey *et al.* Cooley (*8*), in 1929, found the same organism on stocks in Tennessee greenhouses. Kendrick (*11*) reported a bacterial disease of stocks in California as early as 1933 and considered the pathogen to be closely related to *Phyt. campestris* morphologically, but to differ in pathogenicity. In 1938 Wilson (*19*) reported a disease of stocks

[1] Received for publication August 15, 1941.

[2] Associate Professor of Plant Pathology and Associate Plant Pathologist in the Experiment Station.

[3] Assistant Professor of Plant Pathology and Assistant Plant Pathologist in the Experiment Station.

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[5] For explanation of the spelling "Mathiola" see page 8 of citation 2 in "Literature Cited."

[6] Italic numbers in parentheses refer to "Literature Cited" at the end of this paper.

[7] Discussed under the name *Cheiranthus annuus* or Zomerviolieren, terminology used in the European horticultural trade for summer stocks. Misunderstanding of this usage of the name possibly has been responsible for reports in literature compilations of the occurrence of both *Phytomonas campestris* and *Bacterium matthiolae* on wallflower (*Cheiranthus* sp.). The writers are indebted to Mr. G. C. Groenewegen for this information.

in New South Wales to be probably the same as that described by Kendrick. Malençon and Delécluse (*12*) found *Phyt. campestris* on stocks in Morocco in 1934.

Cooley (*8*), and von Faber (*9*), observed the similarity of the causal organism to *Phytomonas campestris,* but did not report cross-inoculation studies. Wilson (*19*) noted morphological and physiological similarities of the stock organism to *Phyt. campestris,* but it failed to infect other crucifers.

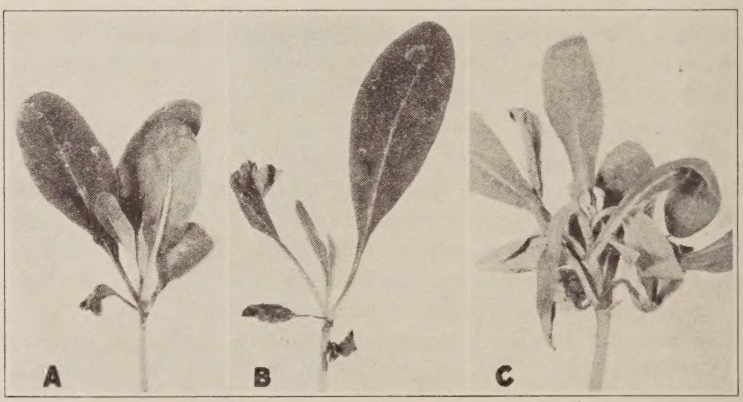

Fig. 1.—Seedling plants grown from diseased seed in sterile soil: *A,* showing one collapsed cotyledon and the lesion on main stem at base of cotyledon petiole 26 days after planting; *B,* a more advanced stage showing both cotyledons collapsed, with lesion at base of cotyledon petiole, and wilting of primary leaf on the left; *C,* a seedling plant from a field planting, showing wilting and collapse of the central growing point because of internal bacterial infection.

In 1912 Briosi and Pavarino (*3, 4*) described a bacterial disease of stocks in Italy and named the pathogen *Bacterium matthiolae.* This same organism was reported by Rudorf and Job (*16*) as attacking stocks in Argentina in 1931. Adam and Pugsley (*1*) found a bacterial disease of stocks in Victoria, Australia, in 1933, but did not identify the pathogen with either *Phytomonas campestris* or *Bact. matthiolae.* Burkholder (*6*) considered that the organisms described by Briosi and Pavarino and by Adam and Pugsley were *Phyt. syringae* (van Hall) Bergey *et al.,* since these organisms did not differ markedly from *Phyt. syringae* culturally, morphologically, or in pathogenicity. In Italy, Mameli-Calvino (*13*) reported a similar disease as destructive in 1937; Santarelli (*17*) as especially so in 1939. The latter agreed with Burkholder that the organism was properly *Phyt. syringae,* rejecting the binomial *Bact. matthiolae.* This disease has not been reported in the United States but has been experimentally produced here (*6*).

SYMPTOMS

The disease of stocks primarily attacks the vascular system of the main stem and lateral branches, and often extends into the leaf petioles and seed peduncles. The cortical tissues usually also are involved; and small to large irregular linear dark-green water-soaked areas show on the main stem and lateral branches, which turn dark brown with age and show open fissures or cracking (figs. 3 and 4, *B*).

Fig. 2.—Symptoms of bacterial blight on young field-grown stocks: *A*, dark lesion at the base of a young lateral shoot; *B*, dark sunken lesions at old leaf scars on the lower part of the stem of the plant; *C*, girdling of the lower stem as a result of leaf-scar invasion.

Under field conditions the disease is first evident as sudden wilting and collapsing of the young plants when they are 2 to 4 inches high (fig. 1, *C*). Close examination shows the main stem to be soft and water-soaked from the ground upward, sometimes into the growing tip. The stem tissues are yellowish, soft, and mushy. Under very moist conditions yellowish drops of exudate often appear along the stem. The nearness of the seedlings favors rapid spread of the disease from one plant to the next in the row under high-moisture conditions and predisposes to the long linear areas of dead plants often observed. The small collapsed plants soon dry up and disappear, often being covered by later cultivation. After the

first severe appearance of the disease on the seedling plants and the subsequent disappearance of the dead plants, the fields have the appearance of a poor, irregular stand.

Under greenhouse conditions the disease attacks the seedlings soon after they emerge from the soil and is first evidenced by the wilting and collapse of one or both cotyledons and by a water-soaked appearance of the stem at the base of the cotyledon petioles (fig. 1, *A* and *B*). It quickly

Fig. 3.—Severe stem lesions and longitudinal cracking of the cortical tissue on a young stock plant.

invades the growing tip, and often causes a collapse and death of the young plants like that observed under field conditions.

After the surviving plants have attained some size and the stems have become woody, the disease progresses less rapidly. Primary infection then usually shows on the older plants at old leaf scars near the ground line as a dark, water-soaked area around the leaf scar and sometimes extends up the stem for several millimeters (figs. 2, *B* and *C;* 4, *A* and *C*). Often all the leaf scars for 2 to 3 inches up the stem from the ground may show infection, and the entire stem soon becomes girdled (figs. 2, *B* and *C;* 4, *C*). There is a noticeable yellowing and dropping of the lower leaves. Severely diseased plants wilt and die, or are broken off at the

ground by wind, or may continue to grow feebly, the cortical tissue remaining intact along one side of the plant. Older and more woody plants often are encircled by a somewhat sunken and darkened infected area extending from the ground 2 to 4 inches up the stalk, with irregular

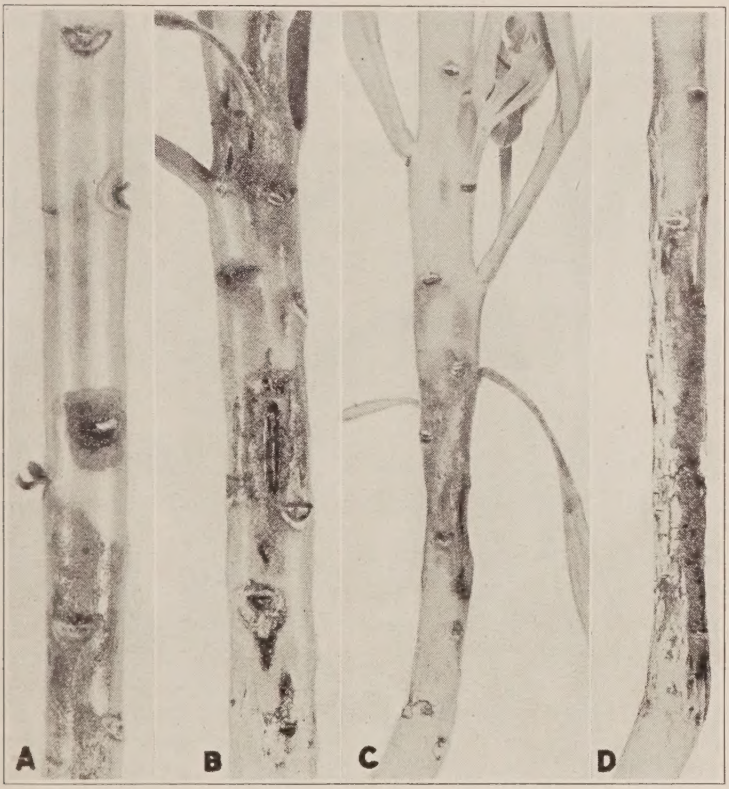

Fig. 4.—Different stages of bacterial blight on the main stems of field-grown stock plants: *A*, young lesions at the leaf scars, showing dark, water-soaked areas; *B*, a more advanced stage of leaf-scar infection and longitudinal cracking of old cankers; *C*, severe canker showing girdling and shrinking of the stem 3 inches above the ground line; *D*, girdling of the stem and irregular cracking of the cortical tissue.

cracking of the cortical tissue (fig. 4, *D*). Infection progresses up the plant in the vascular system and is usually localized in the nodal tissue around the petiole base (fig. 4, *A* and *B*). The leaves, having turned dull yellow, may drop from the plant, leaving a dark-brown cortical infected area around the leaf scar; or they sometimes wilt, droop, and remain attached. In older and larger plants, irregular, linear, dark-green to

brown surface lesions appear on the stem (figs. 3 and 4, *B*) and on the lateral branches, even extending into the racemes and pedicels (fig. 5, *A*). Many of the small branches are girdled and killed. Often the central terminal branch is so severely diseased as to produce no seeds, whereas

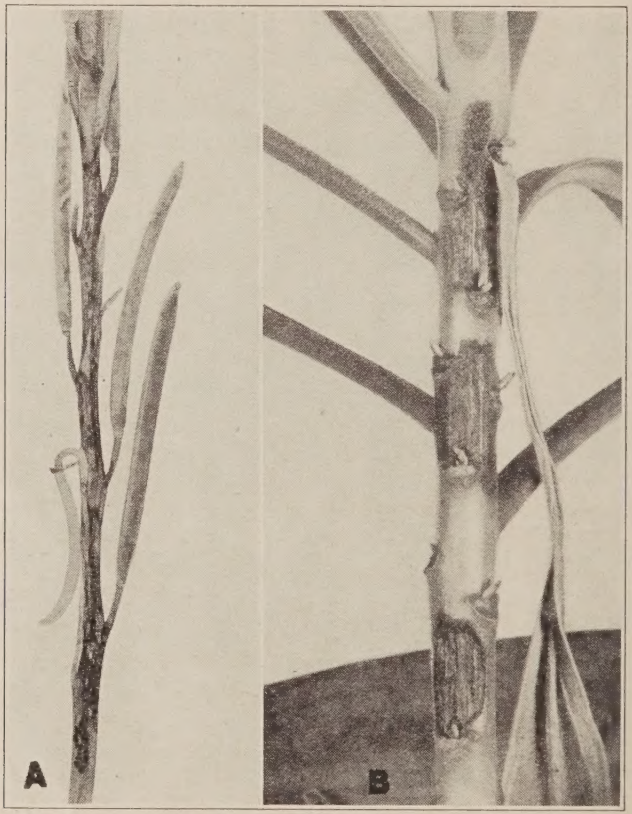

Fig. 5.—*A*, Severe lesions on a lateral branch, showing the lesions extending into the pedicel. *B*, Lesions on the main stem, resulting from artificial inoculation after slight wounding of the tissue at the leaf base.

later lateral branches show less severe infection and produce a fair quantity of seed.

Terminal and lateral branches often show long dark-brown sunken lesions without any macroscopically apparent vascular connection with lesions on the main stem below. Such lesions sometimes extend into the seed-pod pedicel (fig. 5, *A*) and in a few cases have been observed on the seed pods. To judge from macroscopic sections of diseased plants, the bac-

teria travel up the vascular system from the lower leaf-scar infection centers and become localized in pockets in the vascular tissues, causing large, sunken, dark brown surface lesions on main stem and the upper branches.

In California the most characteristic and noticeable symptom of the disease is the dark discoloration around the leaf scars on the main stem (figs. 2; 4, *A*, *B*, and *C*). This characteristic apparently distinguishes the disease from that caused by *Phytomonas syringae,* in which the stem lesions are not described as centering on leaf scars.

Definite leaf spots have not been observed in the field nor produced in the greenhouse by atomizing a bacterial suspension on the plants. This fact further differentiates this disease from that described by various workers in Italy, by Adam and Pugsley (*1*) in Australia, and by Rudorf and Job (*16*) in Argentina, since all these observers report water-soaked leaf spots that later turn brown.

Symptoms have not been observed on the root systems of diseased plants nor have they been reported by other investigators.

ECONOMIC IMPORTANCE

In California the damage has thus far been greatest to the seed crop. The seeds are usually planted in the field during November. The disease becomes evident on the young plants early in the spring; and under high-moisture and moderate-temperature conditions, entire blocks of plantings are killed. Under less favorable climatic conditions, long sections of rows are destroyed. Thus the primary damage is a serious reduction or total loss of stand. Since the disease continues to spread and develop throughout the life of the crop, those plants surviving the early attack of the disease eventually may be killed or may produce little or no viable seed. The damage may range from a trace in some plantings to a total loss in others. With favorable climatic conditions and severe seedling infection, the disease usually results in a total loss of the seed crop; it is undoubtedly the limiting factor in commercial stock seed production in California.

This blight has been observed in home gardens and outdoor commercial cut-flower plantings in California, and markedly reduces the number and quality of the racemes. The diseased, partially defoliated appearance of infected plants as well as the death of many plants makes them objectionable in home plantings.

The disease apparently is very destructive in other regions also. It is reported as causing heavy losses in New South Wales (*14, 19*), killing 12,000 seedlings in one nursery alone in 1939. According to Cooley (*8*), it occasioned severe injury in Tennessee greenhouses in 1929 and 1930.

CHARACTERISTICS AND DESCRIPTION OF PHYTOMONAS INCANAE N. SP.

Microscopic examination of thin, freehand cross sections of diseased tissue in a drop of water showed abundant oozing of bacteria from the cut edges. Repeated isolations from diseased tissue by the dilution-plate method constantly yielded a yellow bacterial organism closely resembling *Phytomonas campestris* (Pammel) Bergey *et al.* in macroscopic characters.

Cultural Characters.—For detailed cultural character studies, four different isolates were carried in parallel series with a culture of *Phytomonas campestris* from cabbage. Unless otherwise stated, all culture media were prepared according to methods prescribed by the Committee of the Society of American Bacteriologists for the study of pure cultures (*18*).

Small bacterial colonies were visible on dilution plates of neutral beef-extract agar and neutral potato-dextrose agar in 24 to 36 hours. In 48 to 60 hours they were circular, slightly raised, light glistening yellow, and gradually became pale yellow with age. Growth on neutral beef-extract agar slants was moderate, filiform, slightly raised, glistening, and smooth, and turned from picric yellow to primuline yellow (Ridgway, *15*) with age. The growth on neutral potato-dextrose agar slants was slightly more vigorous than on beef-extract agar slants and changed from picric yellow to amber color with age, but otherwise exhibited the same general character of growth.

Four types of media were used to determine acid production from carbohydrates: synthetic agar, synthetic broth, beef-extract agar, and beef-extract broth. All cultures were inoculated with a 2-millimeter loop of a heavy suspension of bacteria in water and held at room temperature. Bromthymol blue was added to each medium, and the reaction adjusted to produce a grass-green color. Alkaline production caused the medium to change to a darker-green to blue color.

The results secured with the four isolates of the stock organism and *Phytomonas campestris* were fairly uniform in the synthetic agar and broth media. All cultures produced acid from the following carbohydrates: dextrose, lactose, sucrose, mannite, d-galactose, xylose, d-mannose, raffinose, glycerine, and trehalose. Though the stock isolates failed to produce acid from maltose, *Phyt. campestris* produced strong acid in both the solid and liquid media. No growth occurred in the synthetic media containing rhamnose and l-arabinose except with *Phyt. campestris* on the solid media containing l-arabinose, where strong acid production was noted. The results obtained with the same carbohydrates in beef-

extract agar and beef-extract broth containing peptone were at variance with those obtained in the synthetic media. The stock isolates produced acid only in the liquid media containing dextrose and sucrose and in beef agar containing l-arabinose. *Phyt. campestris* produced acid in the beef broth containing dextrose, maltose, sucrose, and d-galactose, and in the beef agar with l-arabinose. In all other cultures the color change indicated weak to strong alkali production. The results obtained with the carbohydrate compounds in the beef-extract media agree with those of Burkholder (*5*), who states that the members of the *campestre* group produce a strong alkali and neutralize any acid formed when a protein digest, such as peptone and beef extract, is added to the medium.

In the acid-production tests from carbohydrates, the four isolates of the stock organism and *Phytomonas campestris* agree with the exception of acid production by *Phyt. campestris* from maltose and l-arabinose in the synthetic media.

No gas was produced from lactose, maltose, sucrose, d-mannite, glycerine, or potassium nitrate in nutrient broth. Gelatin was liquefied and milk digested, leaving a cloudy turbulent liquid. Indol production was negative, and nitrates were not reduced. The organism tolerated 3 per cent sodium chloride in neutral broth and grew in beef broth of pH 4.4 to 4.7. Growth was slight in Fermi's solution, absent in Cohn's solution, and good in Uschinsky's solution without pigment formation. No reduction of starch was evident when agar cultures containing starch were flooded with an alcoholic solution of iodine.

The organism is a short rod (0.6μ to 2.5μ by 0.4μ to 0.8μ), motile by a single polar flagellum (fig. 6, *B*), Gram-negative nonsporeforming, acid-fast, and aerobic, and stains readily with gentian violet and carbol fuchsin dyes.

The morphological and cultural characters of the stock organism do not differ greatly from those of the black-rot organism, *Phytomonas campestris*. The main morphological difference is a slight variation in cell measurement; the black-rot organism averages 0.7μ to 2.2μ by 0.4μ to 0.7μ, and the stock bacterium averages 0.6μ to 2.5μ by 0.4μ to 0.8μ. The principal culture-character differences were the production of acid from maltose and l-arabinose, the production of indol, and the diastatic action of starch by *Phyt. campestris* in the parallel series of cultures.

These differences alone might not warrant the creation of a new species. Since, however, pathogenicity studies show *Phytomonas campestris* and the stock bacterium to be entirely different, it seems desirable to consider the latter a new species. The name *Phytomonas incanae* n. sp. accordingly is designated herein for the organism responsible for the bacterial blight disease of garden stocks.

Technical Description.—The technical description of the organism follows:

Phytomonas incanae n. sp., cylindrical rods, rounded at ends, single or in pairs; single cells 0.6μ to 2.5μ by 0.4μ to 0.8μ (average, 1.33μ by 0.62μ); motile by one polar flagellum; aerobic, nonsporeforming; Gram-negative. Surface colonies on beef extract and potato-dextrose agar are round, smooth, convex or pulvinate, glistening, margin entire, picric yellow to amber color. Gelatin liquefied, milk cleared without coagulation; nitrates not reduced; no indol; no gas produced from lactose, maltose,

Fig. 6.—*A*, Stained microtome section of infected stock seed, showing bacteria in the funiculus as a dark granular mass just beyond the point of the arrow. *B*, Bacterial cells showing a single polar flagellum.

sucrose, d-mannite, or glycerine; acid produced from dextrose, lactose, sucrose, mannite, d-galactose, xylose, d-mannose, raffinose, trehalose, and glycerine; no acid from maltose, l-arabinose, or rhamnose. No growth in Cohn's solution; growth in Fermi's and Uschinsky's solutions, without pigment formation.

Pathogenic on stems and petioles of *Mathiola incana* R. Br.

Type locality: Monterey County, California.

Pathogenicity Studies.—The pathogenicity of the organism in question has been repeatedly demonstrated by greenhouse inoculations. Inoculations were made by spraying a water suspension of bacteria from an agar culture upon young plants growing either in pots or in greenhouse flats. In some cases the stems were wounded slightly at the leaf axil with

a dissecting needle; in others no wounds were made. Inoculated plants usually were placed in a large, moist chamber in the greenhouse to prevent rapid drying.

In fourteen inoculation trials where the stock bacterial organism was sprayed on young stock plants and slight wounds made on the stem, 39 out of 40 plants showed typical stem infection (fig. 5, *B*). Twenty trials conducted by spraying the organism on the plants without wounding showed typical stem and petiole infection on 168 out of 283 plants. In four trials in which suspensions of *Phytomonas campestris* were sprayed on young stock plants, the stems of which were then wounded, the 24 inoculated plants remained healthy. In the same number of trials sprayed without wounding, the 25 inoculated plants also failed to show the disease.

In five trials involving 19 young, wounded cabbage plants sprayed with the stock organism, no disease developed; and likewise in six trials involving 33 unwounded cabbage plants sprayed with inoculum no disease developed. In three trials in which 15 young cauliflower plants were sprayed with the stock organism, no disease developed. Seven trials with 41 young cabbage plants sprayed with *Phytomonas campestris* resulted in 29 cases of disease; and in three trials with cauliflower plants sprayed with *Phyt. campestris*, 13 out of 18 plants developed typical black rot.

As shown by the results of reciprocal inoculation tests with stock, cabbage, and cauliflower plants, the stock organism will not infect either cabbage or cauliflower plants, and *Phytomonas campestris* will not go to stock plants. In all reciprocal inoculation trials the stock organism produced typical infection on the stock plants, as did *Phyt. campestris* on cabbage and cauliflower. This leaves little doubt that the two organisms are distinct in their pathogenic relations. Wilson (*19*), working with apparently the same bacterial disease of stocks in New South Wales, failed to infect cabbage, cauliflower, Swede turnip, radish, or wallflower with the stock organism in question. In California, black rot of crucifers caused by *Phyt. campestris* is rare and unimportant. Another point of distinction, therefore, for the species *incanae* is its extreme destructiveness to stocks even on the same farms where no black rot of cabbage is observed.

TRANSMISSION BY SEED, EXTERNALLY AND INTERNALLY

Early in these studies it appeared probable that the disease originated primarily from the seed since among large blocks of different types of stock plantings in the same field, certain blocks showed severe disease, whereas others showed no disease or only scattering infections.

In May, 1936, a close examination of severely diseased plants in the field showed water-soaked lesions on the branches of the racemes and the pedicels (fig. 5, *A*). Many of the seeds in such siliques were found embedded in a yellow, gummy exudate. Siliques and attached pedicels from these severely diseased branches were brought into the laboratory for isolation. In one trial, tissue cultures from 8 out of 23 pedicels showed typical yellow bacterial growth. Out of 70 seeds removed aseptically from the 8 siliques that showed bacterial growth, 23 from 7 siliques developed the same type of bacterial colonies secured from the pedicels. The pathogenicity of the bacteria from the pedicels and seed was proved by inoculation tests.

As microscopic examinations and isolation tests revealed, the bacteria were in the vascular system of all parts of the plant. Since they readily invaded the vascular system of the pedicel, it seemed possible that seed invasion also might occur. Mature green seeds from diseased siliques were fixed in a solution of acetic acid, formalin, and corrosive sublimate and then embedded in paraffin. Stained microtome sections showed the bacteria in the funiculus of the seed (fig. 6, *A*).

In the fall of 1936 seed was harvested by hand from diseased plants in a commercial field. It was divided into three lots as follows: (1) seed from individual racemes showing severe stem and pedicel lesions, (2) seed from lateral branches showing severe stem lesions, and (3) seed from entire plants showing all stages of the disease. All three types of seed were harvested by beating the racemes in a cloth bag to shell out the seed.

On December 26, 1936, four flats of sterile soil were planted with the seed harvested in September. Because these flats were held in a greenhouse without steam heat, germination and growth were relatively slow. Early examination did not show any disease. The flats were held in the greenhouse until April, 1937, when a close examination of the large plants (4–10 inches tall) showed an abundance of bacterial blight. Two flats planted from diseased seed lot 1 showed 33.2 per cent of 271 plants diseased. The two flats planted from seed lot 2 showed 42.4 per cent of 438 diseased. Almost certainly the high percentage of disease herein recorded resulted from secondary infection. As subsequent tests revealed, the disease appears when the plants are small (fig. 1, *A* and *B*) and infected plants might easily have been overlooked. Where the plants are growing under crowded conditions, the stunted diseased plants soon are overshadowed by the more rapidly growing healthy plants and may be easily overlooked unless critically examined. While these tests obviously did not give accurate data on the percentage of seed transmission, they did show that the disease is carried by the seed.

EXPERIMENTS IN CONTROL METHODS

Effect of Hot-Water Seed Treatment on Seed Germination.—Seeds of ten-week stock, variety Crimson, from a commercial lot were treated with hot water to determine how such treatment affects germination. The seeds were enclosed in small cheesecloth bags, immersed in water at the desired temperature for a definite period, removed, and cooled at once by plunging into cold water. They were then spread in a thin layer on the cheesecloth and dried within a few hours. When thoroughly dry they were placed on moist filter paper in a moist chamber and held at 25° C.

TABLE 1

Effect of Hot-Water Treatment on Germination of Seed of Ten-Week Stock, Variety Crimson

Temperature, degrees centigrade	Time in minutes	Number seeds planted	Per cent germination
50	10	200	90.5
52	10	200	89.5
52	20	200	75.5
53	10	200	91.5
54	10	200	87.0
55	10	200	85.0
60	10	200	0.0
65	10	200	0.0
Control	10	600	90.8

Table 1 summarizes the effect of hot water on germination. As the table shows, a water bath from 50° to 55° for 10 minutes had no significant effect on seed germination; a temperature of 60° or above was lethal.

Hot-Water Seed Treatment as a Control Method.—On April 9, 1937, seeds from lots 1, 2, and 3 were treated in the hot water at 53° C for 10 minutes, dried, and planted in flats of sterile soil along with similar flats of untreated seed. Single seeds were spaced at 1-inch intervals. Twenty-one days later the plants showed infection, and on May 10 final counts were made (trial number 1, table 2).

On September 25, 1937, seed from lots 2 and 3 were planted in flats of sterile soil from seed that had been treated on June 21, 1937, with hot water for 10 minutes at 53°, 54°, and 55° C. The seedling disease appeared in 26 days from planting, and counts were made. No new cases appeared the following week. Data secured at the end of one month (trial no. 2, table 2) represent seed transmission, since the plants were sufficiently spaced and were small enough to preclude spread from plant to plant. No serious injury to germination occurred in any of these treatments.

The data presented in table 2 show that a relatively small number of seeds carry the causal organism and that the hot-water seed treatment entirely eliminated the disease in three out of five trials. In the other two trials, the amount of disease surviving the treatment was greatly reduced.

On May 28, 1940, seed of four commercial varieties was treated at 53°, 54°, and 55° C. Samples taken from the indicated varieties of the large lots treated at 53° in November, 1939 (table 4) also were used. All seeds were planted in greenhouse flats on May 30, 1940, and data were taken periodically up to October 28, 1940 (table 3).

TABLE 2

SUMMARIZED RESULTS OF HOT-WATER SEED TREATMENTS, SHOWING EFFECTIVENESS IN CONTROL OF THE DISEASE

Seed lot	Trial no.	Age of seed in days	Treated with hot water at 53° to 55° C		Untreated check	
			Number of plants	Per cent of plants diseased	Number of plants	Per cent of plants diseased
1	1	210	207	2.4	325	12.3
2	1	210	255	0.4	570	3.7
3	1	210	397	0.0	361	14.1
2	2	270	327	0.0	116	0.8
3	2	270	410	0.0	119	1.6

This test also showed that the hot-water treatment at 53° to 55° C for 10 minutes had no significant effect on seed germination and materially reduced or completely eliminated seed infection.

Field Tests of Hot-Water Seed Treatment.—The hot-water seed treatment under field conditions has given excellent control of bacterial blight. In the fall of 1936 one seed grower treated, at 53° C for 10 minutes, about 40 lots weighing 30 pounds. From this treated seed 17 acres were planted that fall; but severe weather unfortunately prevailed, and the entire planting was frozen. A repeat planting also was frozen. Four small block plantings survived the freezing weather, and a close examination of these blocks the following season showed slight disease in two blocks and none in the other two.

In the fall of 1937 a small lot of hot-water-treated diseased seed with an untreated lot for control was planted in Monterey County. In June, 1938, the plants from treated and untreated seed were carefully examined and counted. Of 492 plants from treated seed 6.7 per cent showed disease; whereas of 528 plants from the untreated seed 42.2 per cent showed disease. Evidently, again, there had been some secondary spread

in this test, as there usually was more than one diseased plant in a place; often two or three diseased plants were grouped together.

Again in the fall of 1938 a small lot of diseased seed was treated with hot water at 53° C for 10 minutes and planted in a commercial field with an untreated control. On March 15, 1939, after many days of rainy

TABLE 3

EFFECT OF HOT-WATER TREATMENT OF DISEASED STOCK SEED AT VARIOUS TEMPERATURES ON PLANT EMERGENCE AND INCIDENCE OF DISEASE IN GREENHOUSE-FLAT TESTS

Type and variety	Untreated check		Treated for 10 minutes at the given temperatures							
			53° C*		53° C		54° C		55° C	
	Per cent emergence	Per cent diseased	Per cent emergence	Per cent diseased	Per cent emergence	Per cent diseased	Per cent emergence	Per cent diseased	Per cent emergence	Per cent diseased
Super Giant Imperial, Dark Blue	53.0	6.6	58.0†	0	44.0	1.1	54.5	0.0	51.0	1.0
Perpetual Branching, Queen of the Belgians	58.0	4.3	65.0†	0	55.5	0.0	38.0†	2.6	58.0	2.6
Perpetual Branching, Purple	70.5	14.2	72.0†	0	82.5	0.6	81.5	0.0	79.5	0.6
Early Giant Imperial, Rose‡	65.5	1.5	51.0†	0	58.0	0.8	73.0†	0.0	46.0	0.0
Early Branching Nice, Monte Carlo	....	....	70.0†	0	.		..	.		

* Seed planted in these trials was from the respective treated lots represented by field plantings in table 4.
† Trials in which 100 seeds were planted; in all others 200 seeds were used.
‡ For this variety seed was from the 1937 crop; for all others from the 1939 crop.

weather, examination and counts were made. From the treated seed 4.8 per cent of 372 plants were diseased, whereas in 371 plants from the untreated seed 70.6 per cent were diseased.

In November, 1939, a large-scale treatment of 125 pounds of seed representing about 85 varieties was made; and from this, 29 acres were planted in Santa Barbara County in mid-December. Except for a few varieties growing in a portion of the field flooded during the winter with runoff from an area in which stocks were severely diseased the year before, the fields in general showed a highly satisfactory control. This condition is reflected in the increased yields, for 60 varieties, over the 1939 crop (table 4).

The greenhouse trials and the field trials show that the disease can

be reduced to a minimum or entirely eliminated by treating the seed at 53° C for 10 minutes. An exposure for 10 minutes at 55° might completely control the disease, but also might result in a slight to moderate reduction in germination. Since, however, the disease spreads rapidly under field conditions, the reduction in germination from the 55° treatment probably would be less damaging to yield than the ultimate loss from disease spreading from a few scattered infection centers.

TABLE 4

AVERAGE YIELD OF SEVERAL TYPES OF STOCKS ON A RANCH IN 1939 WHEN BACTERIAL BLIGHT WAS SEVERE AND IN 1940 WHEN HOT-WATER TREATMENT OF SEED WAS PRACTICED

Type	Number of varieties	Average seed yield per acre (pounds)*	
		1939—no seed treatment used	1940—hot-water seed treatment used
Ten Week	15	60.1	147.7
Early Branching Nice	11	86.2	159.5
Super Giant Imperial	3	48.0	47.3†
Early Giant Imperial	14	130.9	187 6
Perpetual Branching	5	29.6	84.8
Column	12	20.2	234.2

* That the yield differences for the two years were largely a result of control of the disease was evident from field observations. The 1940 season in these fields was about as favorable for the disease as that of 1939, as shown by losses of varieties of the Super Giant Imperial type and severity of attack of occasional plants in other types of stocks.

† In area flooded from previous year's severely diseased planting; ineffectiveness of treatment a result of soil contamination.

Seed-Treatment Methods.—Hot-water treatment of stock seed is rendered somewhat difficult by their tendency to swell and become gelatinous in hot water, a condition that results in slow drying. The following method has made the process sufficiently easy and dependable to be used by commercial growers without difficulty: Porous but strong cheesecloth is cut into squares large enough so that the seed being treated in a given lot can be spread on the cloth to about single-seed thickness. For a handful of dry seed, cloth a yard square is satisfactory. The seed is placed in the middle of the square, and the cloth securely tied to form a loose bag. If tied into a tight ball the seed will swell and perhaps rupture the bag, in addition to making difficult rapid heating throughout the mass. Each bag can be labeled and as the seeds remain on it throughout the treatment, the hazard of mixing is reduced.

The bags are plunged into a large tank of water at 53° to 55° C (127.5° to 131° F) and immediately kneaded with the fingers to drive out the air and to aid penetration of water. If they are not kneaded, only the

outer layers of seeds become wetted, and uniform heat penetration is impeded. The volume of water should be large (100–200 gallons), and there should be some means of maintaining the temperature within the range indicated; running hot water into the tank or use of gas burners under it have been equally satisfactory. The water must be continuously agitated to equalize the effect of surface cooling of the tank.

After exactly 10 minutes the bags are removed, plunged immediately into cold water, and again kneaded to facilitate rapid, uniform cooling. When cool they may be squeezed by hand to expel excess water. Wringing should be avoided because in some varieties it causes the seed coat to slip off.

Since the chaff from untreated seed scatters in handling, the initial sacking operation should not be done in the room where the treated seed is drying. The cooled bags should be placed on newspaper spread out on the ground, on tables, on tops of cold frames, or similar areas. The paper takes up some of the excess water and also protects the seed from recontamination. The wet seeds are spread out by hand, for the use of any hard object would injure them. The seeds slip over one another and adhere to the cloth; this gives essentially a single layer. The cloth then may usually be handled without fear of the seeds dropping off; otherwise, a short time on the paper will dry them sufficiently. For small lots treated on bright sunny days, spreading the cloths on dry newspapers, with perhaps a fan blowing across them, will dry them within a few hours. If large lots are involved and if space is at a premium, the cloths with the adhering seeds may be hung on lines in a sunny, airy place where they will dry quickly. In a windy location it may be necessary to hang weights on the bottom of the cloths to prevent whipping. The seed should be dried in 4 to 6 hours without the use of heat. Care should be exercised to avoid cooking the seed by placing it to dry on very hot objects or in the open sun on very hot days.

When thoroughly dry, the seed is easily removed by crumpling the cloth in the hands and by gently rubbing the crumpled parts together. The seed may then be stored in the same cloth or placed in seed bags. Old bags, if used, should be sterilized by boiling. The seed may be separated from the crusts formed in drying by being placed on a screen just large enough to pass single seeds. After sifting out the singles, one can break up the residue readily by hand-rubbing against the screen.

Such treated seeds can be stored for at least several months without seriously reducing germination. It is advisable to make germination tests before planting in order to determine the requisite density of sowing.

It hardly seems advisable for seed companies to treat stock seed for the general market. An equivalent amount of labor applied in careful

treatment of the seed planted for commercial production, coupled with careful roguing of the fields and burning of any infected plants, should make wholesale treatment unnecessary. Perhaps in some high-priced types, such as column and other florist strains, such treatment might prove advisable. Until field control has been effectively applied, however, such a program is not basically sound. Greenhouse growers probably would be justified in treating the seed before planting, but ordinarily the home gardener should not attempt this.

Soil Treatments.—At present it is not known how long the stock bacterium will survive in the soil. To judge from abundant field evidence, however, 75 per cent of the plants from treated seed may be infected by replanting a given area to stocks in the next cycle (that is, within 4 months). Stocks should not be grown in an area subject to flooding with drainage water from an area of a diseased previous cycle. Heavy losses have been sustained with stocks in California and with cauliflower in New York (*7*) from such contamination. Land-leveling operations also may spread contamination in the same general way.

Clayton (*7*) found that *Phytomonas campestris* survived the winter in field soil in New York, but that healthy plants remained disease-free in such soil after a one-year rotation. The bacteria did not survive in dead cauliflower trash. In California it would seem advisable to burn the trash from diseased fields of stocks after harvest and to practice at least a two- or three-year rotation until more definite information is available.

In the home garden or greenhouse, infested soil may be treated if a continuous planting of stocks is desired. Steam sterilization or a soil drench, some weeks before planting, with enough 1–50 solution of formaldehyde to penetrate to a 6-inch depth has proved satisfactory for the stock bacterium (*14*) and for the cabbage black-rot organism (*7*).

There are indications that, under California conditions, late planting of stocks greatly reduces losses from this disease. Whether the cause is the decreased rainfall in the spring, a generally hardier growth condition or other factors, is not known.

Although there are indications of varietal differences in susceptibility to this disease, present observations do not warrant listing them at this time.

SUMMARY

In California a bacterial blight of garden stocks has occurred in commercial seed plantings in coastal areas since 1933. It also has been observed in home gardens and in cut-flower plantings.

The disease attacks the main stem and lateral branches in all stages of the plant's development. Seedlings show a soft, water-soaked condition of the main stem and growing tip and a general collapse of the plant.

On older plants, dark, sunken lesions appear on the main stem and the lateral branches. Lesions on the lower stem usually center on old leaf scars and may result in girdling and killing.

The principal damage has been a serious reduction in seed production during certain seasons. Many plants, however, are killed or greatly stunted in home gardens and commercial cut-flower plantings.

The causal organism, which is easily isolated from diseased tissue, forms yellow colonies on agar culture media, and is a short rod, motile by a single polar flagellum. In cultural and morphological characteristics it closely resembles the organism causing black rot of crucifers (*Phytomonas campestris*), but is distinct in pathogenicity. The causal organism, apparently an undescribed species, is briefly characterized, described, and named *Phytomonas incanae,* n. sp.

The disease has been readily produced under greenhouse conditions by spraying a water suspension of the bacteria on young plants with and without wounding the stem tissue. In reciprocal inoculations with *Phytomonas campestris* and the stock organism on stock, cabbage, and cauliflower plants, the former failed to produce the disease on stock plants and the stock organism was nonpathogenic to cabbage and cauliflower.

The bacteria invade the vascular system and have been recovered from the stem, lateral branches, peduncles, and seed. Stained microtome sections of mature seed showed the bacteria in the funiculus of the seed. Seed from diseased plants when planted in steam-sterilized soil showed a small percentage of diseased plants.

In experimental tests, immersion of stock seed in water at 53° to 55° C (127.5° to 131° F) for 10 minutes, followed by prompt cooling, resulted in complete control or greatly reduced the incidence of seedling infection under greenhouse conditions without material injury to the seed. The hot-water treatment in field practice greatly reduced the occurrence of the disease and materially increased seed production.

Seed may be successfully treated by enclosing a small amount in a loose cheesecloth bag, immersing in water at 53° to 55° C for 10 minutes, cooling rapidly after treatment, and spreading in a thin layer on the cheesecloth to dry.

Observational evidence indicates that the organism persists in the soil. A two- to three-year rotation should be practiced. Drainage water from an infested area will carry the disease into uninfested fields.

Infested garden or greenhouse soil can be sterilized with steam or by soaking the soil to a depth of 6 inches with a 1–50 solution of formaldehyde.

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